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Development and validation of a gas chromatography–negative chemical ionization tandem mass spectrometry method for the determination of ethyl glucuronide in hair and its application to forensic toxicology☆

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ABSTRACT

Ethyl glucuronide (EtG) is a minor and direct metabolite of ethanol. EtG is incorporated into the growing hair allowing retrospective investigation of chronic alcohol abuse. In this study, we report the development and the validation of a method using gas chromatography–negative chemical ionization tandem mass spectrometry (GC–NCI-MS/MS) for the quantification of EtG in hair. EtG was extracted from about 30 mg of hair by aqueous incubation and purified by solid-phase extraction (SPE) using mixed mode extraction cartridges followed by derivation with perfluoropentanoic anhydride (PFPA). The analysis was performed in the selected reaction monitoring (SRM) mode using the transitions m/z 347 → 163 (for the quantification) and m/z 347 → 119 (for the identification) for EtG, and m/z 352 → 163 for EtG-d_5 used as internal standard. For validation, we prepared quality controls (QC) using hair samples taken *post mortem* from 2 subjects with a known history of alcoholism. These samples were confirmed by a proficiency test with 7 participating laboratories. The assay linearity of EtG was confirmed over the range from 8.4 to 259.4 pg/mg hair, with a coefficient of determination (r^2) above 0.999. The limit of detection (LOD) was estimated with 3.0 pg/mg. The lower limit of quantification (LLOQ) of the method was fixed at 8.4 pg/mg. Repeatability and intermediate precision (relative standard deviation, RSD%), tested at 4 QC levels, were less than 13.2%. The analytical method was applied to several hair samples obtained from autopsy cases with a history of alcoholism and/or lesions caused by alcohol. EtG concentrations in hair ranged from 60 to 820 pg/mg hair.

1. Introduction

Heavy alcohol consumption has a major impact on public health. Its consumption increases the risk of both health and social problems *via* intoxication and dependence. The development of accurate methods to detect heavy alcohol consumption is therefore indispensable. However, discriminate different types of consumer behavior is still a major concern regarding specificity and sensitivity of existing methods.

Ethylglucuronide (EtG) is a direct metabolite of ethanol. It is formed by conjugation of ethanol with glucuronic acid *via* UDP-glucuronosyl transferases [1], and represents less than 0.06% of the ingested ethanol dose [2,3]. Compared to ethanol, EtG is eliminated more slowly with a half-time of about 2.5 h [4]. Therefore, whereas ethanol is completely eliminated from body, EtG can be detected in blood and urine for up to 8 and 80 h, respectively [4,5]. EtG also accumulates in hair allowing a larger retrospective time window for the alcohol consumption detection. EtG could therefore be considered as a long-term alcohol marker. Furthermore, EtG has been shown of interest as a marker of heavy alcohol consumption [6–9]. Its determination in human hair is therefore of practical interest in clinical and forensic medicine.

EtG analysis requires analytical methods showing highest sensitivity; the concentrations of EtG measured in hair being usually in a pg/mg range. Over the last decades, technical improvements have allowed to increase the sensitivity and several methods have been described in the literature. The most frequently used analytical methods are based on gas chromatography coupled to mass spectrometry (GC–MS) and liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Janda et al. [10] have developed a LC–MS/MS method preceded by a solid-phase extraction (SPE) cleanup step. A limit of detection (LOD) of 51 pg/mg was obtained using 100 mg hair. In contrast, Jurado et al. [11] published a GC–EI-MS method after derivatization with pentafluoropropionic

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anhydride (PFPA). This method showed a LOD of 25 pg/mg after analyze of 100 mg hair. Afterwards, the method sensitivity was improved by the use of SPE extraction followed by GC–MS in negative chemical ionization (NCI) mode as described by Yegles et al. [8], who reached a LOD of 2 pg/mg after analysis of 30 mg hair. More recently, Morini et al. [12] have shown that the use of an API 4000 Q-Trap allows direct extraction solution injection from 100 mg of hair reaching a LOD of 2 pg/mg hair. Finally, one paper reported the use of GC–MS/MS in the electron impact mode after silylation with BSTFA. The LOD was in the same range [13].

GC–MS/MS could be a method of choice for EtG analyses in hair as it combines the advantages of GC (highest separation power) and tandem mass spectrometry (highest selectivity) minimizing or eliminating background interferences; therefore improving selectivity and sensibility. This combination gives a powerful tool for the determination of EtG present at pg/mg levels in hair, only. GC–MS/MS also allows the monitoring of many transitions for identification and quantification of the EtG in compliance with toxicological requirements [14].

Since quality control (QC) allows to evaluate the analytical method reliability, its choice is important. Controls used for method validation are usually constituted by blank hair samples spiked with different concentrations of EtG. These samples do not reflect totally a real hair sample where EtG is incorporated and fixed to the hair matrix [15]. To overcome this disadvantage, control samples have been prepared based on real hair samples. These samples covered a concentration range representative for the routine use and have been tested in a proficiency test by 7 laboratories. To date, from the best of our knowledge, no validation has been done with real hair samples.

So far, all published methods have only been partially validated. The purpose of this study was therefore to develop and to validate an analytical method for the EtG determination in hair providing highest sensitivity and specificity. For this reason, the GC–MS/MS in NCI mode was chosen. The validation consisted in showing that the analytical method is suitable, reliable and accurate for its intended use. Afterwards, the method's applicability was tested on several samples obtained from autopsy cases with a known history of alcoholism and/or pathologies caused by alcohol and reveled by macroscopic and microscopic autopsy finding.

2. Materials and methods

2.1. Chemicals and reagents

Reference standards: methanolic solutions of EtG and EtG-d_5 (I.S.) were purchased from Cerilliant (Round Rock, USA). All chemicals were of the highest analytical grade. Methanol, ammonium hydroxide solution (25%), and formic acid (98%) were from Fluka (Buchs, Switzerland). Acetone and hexane were obtained from Reactolab (Servion, Switzerland) and Merck (Darmstadt, Germany), respectively. PFPA was from Sigma Aldrich (Steinheim, Germany). SPE Oasis MAX (3 ml, 60 mg) cartridges were supplied by Waters (Milford, USA).

2.2. Instruments and GC–NCI-MS/MS conditions

A Varian CP-3800 gas chromatograph (Walnut Creek, CA, USA) coupled to a Varian 1200 L MS/MS triple quadrupole mass spectrometer (Walnut Creek, CA, USA) operating in NCI mode was used for analysis. The system was equipped with a CTC Combi-PAL autosampler (Zwingen, Switzerland).

Compounds were separated on a fused silica capillary column (DB-5MS) with a 5% phenyl–95% methyl-polysiloxane stationary phase (15 m length × 0.25 mm I.D. × 0.25 μm film thickness). The carrier gas was helium with a constant flow of 1 ml/min. Two microliters were injected in splitless mode at an injection temperature of 250 °C. The initial oven temperature of 70 °C was kept for 1 min, increased first at 35 °C/min to 120 °C, then at 5 °C/min to 150 °C, and finally at 70 °C/min to 300 °C. The transfer line was held at 275 °C. Retention times were of 4.65 min for EtG and 4.64 min for EtG-d_5.

Samples were ionized by NCI with methane as reagent gas at a pressure between 8 and 8.5 Torr. The ion source temperature was kept at 150 °C. The GC–MS/MS was performed in selected reaction monitoring (SRM). The precursor ions *m*/*z* 347 for EtG and *m*/*z* 352 for EtG-d_5 were selected in the first quadrupole. The precursor ion

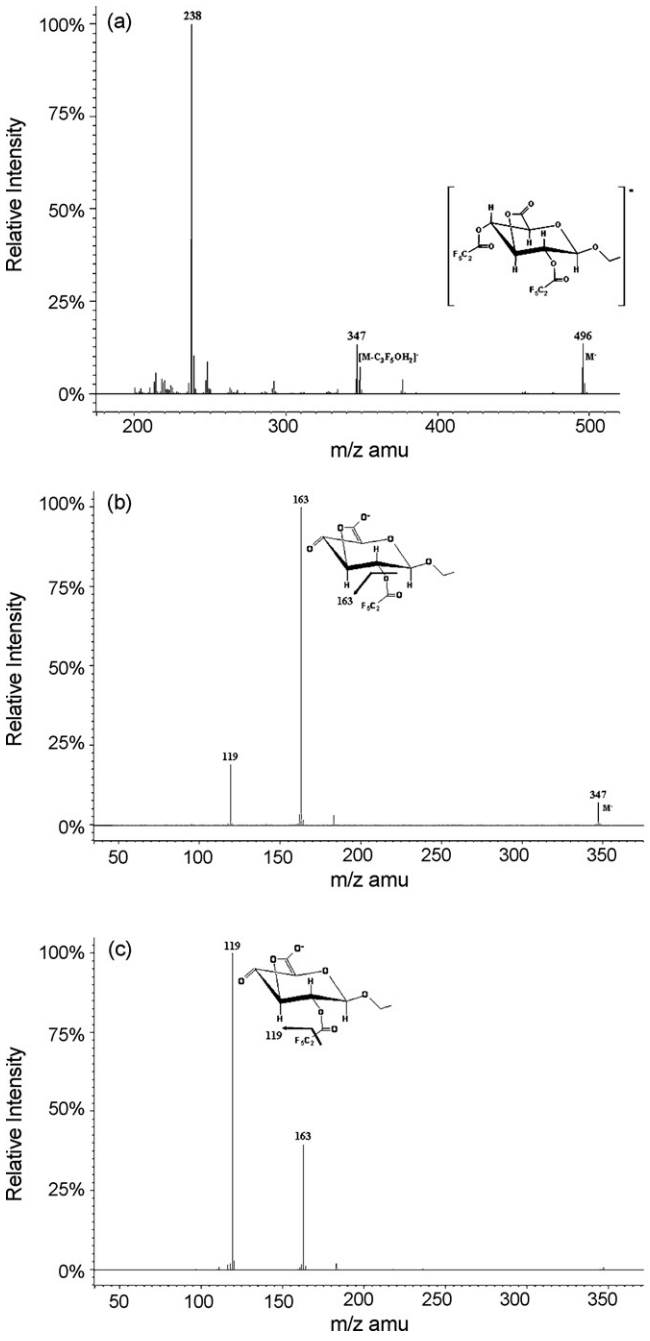

Fig. 1. (a) Negative chemical ion mass spectra of EtG after derivatization with perfluoropentanoic anhydride in full scan mode. (b) Product ion spectra of the PFP derivative of EtG after collision induced fragmentation with collision energy of 5 eV (precursor ion: *m*/*z* 347). (c) Product ion spectra of the PFP derivative of EtG after collision induced fragmentation with collision energy of 25 eV (precursor ion: m/z 347).

m/z 347 should correspond to the molecular ion m/z 496 (shown in Fig. 1a) losing 1 molecule of PFPA followed by a molecular rearrangement resulting in a form stable molecular (see Fig. 1b and c). The precursor ions were chosen for further fragmentation according to their abundance in the mass spectra and their selectivity. The resulting product ions m/z 119 and 163 for EtG and m/z 163 for EtG-d_5 were selected in the third quadrupole after collision in second quadrupole (collision cell) with argon as collision gas at a pressure of 1.3 mTorr. The collision energy was maintained at 25 and 5 eV for the transitions m/z 347 → 119 and 347 → 163, respectively, and 5 eV for the transition m/z 352 → 163. Transition m/z 347 → 163 was retained for the EtG quantification, and transition m/z 347 → 119 was used for the EtG identification. The electron multiplier was set to 1200 V.

2.3. Samples used for validation

2.3.1. Calibration samples (Cal)

Blank hair samples were collected from a teetotaler and were analyzed for the presence of EtG before validation phase. Calibration samples (Cal) were prepared by adequately spiked blank hair powder (30 mg) with appropriate volumes of standard EtG solution (0.1 μg/ml in MeOH) resulting in the concentration range 7-333 pg/mg hair corresponding to 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 ng/30 mg. Twenty nanograms of the internal standard, EtG-d_5 (10 μg/ml in MeOH), were added to each sample.

2.3.2. Quality control samples

QC samples were prepared with hair powder from real autopsy cases with a known history of alcoholism where analyses of biomarkers of alcohol consumption were requested by the authorities. To obtain sufficient quantity of hair, the stock sample was obtained from 2 higher EtG concentration samples. This sample was diluted with hair from 1 teetotaler to obtain 4 QC levels (QC_1, QC_2, QC_3, and QC_4) covering a concentration range representative of routine work. The QC samples were homogenized in a baker during 4 h using a magnetic stirrer.

2.4. EtG proficiency test

Sixteen milligrams of hair powder from the 4 QC levels (QC_1, QC_2, QC_3 and QC_4) were sent to 7 external laboratories. The laboratories had to measure the EtG concentrations in each samples using their own analytical methods. In addition, we recommended our extraction method (see Section 2.7) which was provided with the aliquots.

2.5. Hair sample collection from autopsy cases

Seven hair samples were taken from autopsy cases with a known history of alcoholism and/or pathologies caused by alcohol and reveled by macroscopic and microscopic autopsy finding. Sampling was performed according to the Society of Hair Testing (SOHT) recommendations [16]. Hair samples were collected with scissors from the posterior vertex region of the head, close to the scalp. The samples were then stored at room temperature and kept away from light. The proximal segment (0–3 cm), corresponding to 3–4 months of hair growth, was submitted to the analysis as described in Section 2.7.

2.6. Data acquisition and data analysis

Data acquisition and MS control were realized using the software GC/MS workstation Varian version 6.9. Data were analyzed using analysis of variance (ANOVA) and Grubbs' test.

2.7. Extraction procedure

The hair samples were prepared according to the method of Yegles et al. [8]. Hair was successively washed with deionized water and acetone, respectively for 5 min. The hair was then dried at room temperature and cut roughly with scissors before powdered in a ball mill (Retsch MM 2000, Schieritz, Hauenstein, Switzerland) for 2 min (maximum amplitude). Twenty nanograms of internal standard (EtG-d_5) and 1 ml of distilled water were added to about 30 mg hair powder. The extraction was performed using an ultrasonic incubation for 2 h. After centrifugation at 840 × g for 10 min, the supernatant was applied for cleanup to an Oasis MAX cartridge (3 ml, 30 mg) conditioned with 2 ml methanol and 2 ml deionized water. The cartridge was first washed with 1 ml ammonium hydroxide (NH_4OH, 5%) and secondly with 1 ml methanol. EtG was eluted from the column using 2 ml of a methanol/formic acid (98:2, v/v) solution. The eluate was dried under nitrogen (N_2). The obtained residue was derivatized with 100 μl PFPA for 30 min at 80 °C, dried under N_2, and reconstituted in 50 μl of hexane. Two microliters of extract were injected into the GC–MS/MS system.

2.8. Validation procedure

The validation was done according to the guidelines of the "Société Française des Sciences et Techniques Pharmaceutiques" (SFSTP) [17–19]. The validation parameters were based on the following criteria: selectivity, response function (calibration curve), linearity, trueness, precision (repeatability and intermediate precision), accuracy, LOD and LOQ. Other studied validation parameters were SPE recovery, stability and EtG extraction kinetic from hair. The validation experiments were performed with two kinds of samples: Cal and QC samples over 3 non-consecutive (p = 3) days.

3. Results and discussion

3.1. Selectivity

To evaluate the selectivity of the method, 6 blank hair samples obtained from adult abstainers were analyzed to demonstrate the absence of interference between EtG and endogenous substances. For each monitored transition (m/z 347 → 119 and 347 → 163) no interference peak was observed at the EtG retention time indicating that the method provides satisfying selectivity for EtG analysis. Representative mass chromatograms of one sample are shown in Fig. 2a.

3.2. Response function (calibration curve)

The response function is defined by the relationship between the analyte concentration in the sample and the corresponding response, i.e. the area ratio of EtG to EtG-d_5 within a concentration range [18]. The response function was determined using Cal samples. Three calibration curves were prepared on 3 non-consecutive days (p = 3) at 6 concentration levels (k = 6): 7, 17, 33, 67, 167 and 333 pg/mg of hair; each in duplicate (n = 2). A linear relation was found between the spiked EtG concentration and the measured response in the considered calibration range. To increase the statistical weight of the lowest concentrations, 3 simple linear regression models have been compared: one model based on the least square method and 2 models based on weighted least squares (weighting factors used were $1/x$ and $1/x^2$). The most appropriate model was the linear regression model based on weighted least squares with a weighting factor of $1/x^2$ for EtG. The validation results for the response functions corrected with a weight of $1/x^2$ are presented in Table 1(a).

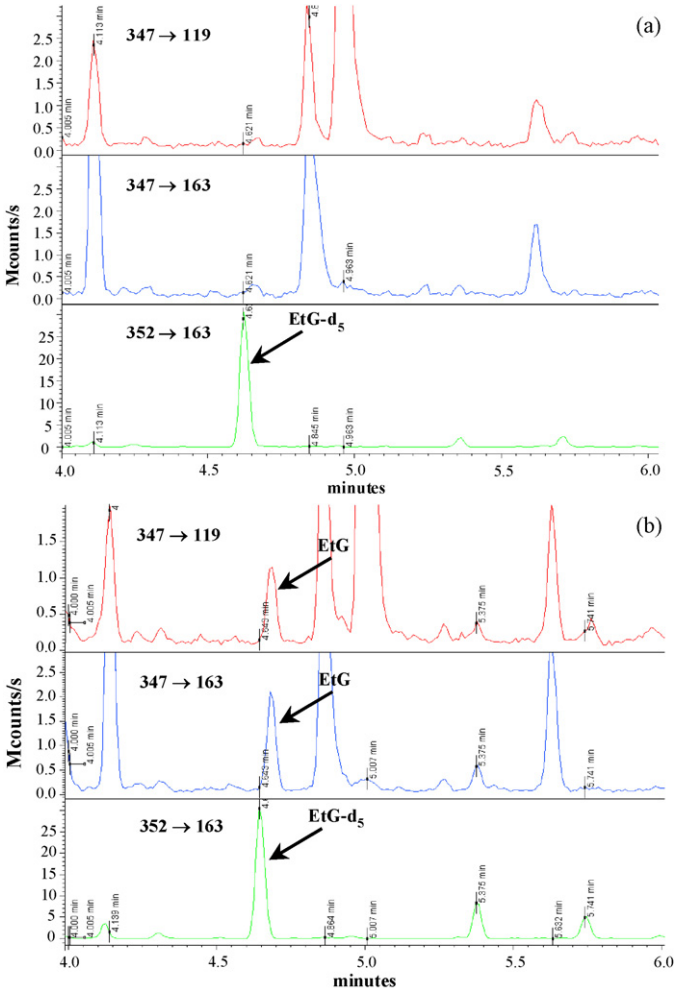

Fig. 2. Representative SRM ion chromatograms of blank hair sample from a teetotaler (a) and a real case close to the LLOQ (b) spiked with 20 ng of EtG-d_5 (internal standard).

3.3. Linearity

The linearity of an analytical method corresponds to its ability, within a given range, to obtain results directly proportional to the analyte concentration in the sample [20]. The linearity was evaluated by fitting the back calculated concentrations of the QC vs. theoretical concentrations by applying the linear regression model based on the least square method. Each non-consecutive day, QC samples were analyzed at 4 concentrations levels ($k = 4$): QC_1, QC_2, QC_3, and QC_4 in quadruplicate ($n = 4$). The lowest concentration (QC_4) was chosen closed to the initially estimated LLOQ prior the validation phase. The QC concentrations were calculated with weighted linear regression ($1/x^2$) using a calibration curve determined at each measurement day. As shown in Table 1(b), a good linearity was obtained with a slope value of 1.014 and a coefficient of linear correlation above 0.999 in the range from 8.4 to 259.4 pg/mg hair.

3.4. Results obtained from proficiency test

EtG concentrations were analyzed on each QC levels by 7 participating laboratories. The laboratories used their own extraction method and the measurements were carried out by GC–MS ($n = 4$) and LC–MS ($n = 3$). Grubb's test was applied on the reported results to exclude potential outlier values, with a risk $\alpha = 5\%$. Based on these results, maximal 2 laboratories had to be excluded partially. The results from the remaining laboratories are shown in Table 2. The mean concentrations were 259.4 ($n = 6$), 130.4 ($n = 5$), 40.8 ($n = 5$), and 8.4 pg/mg hair ($n = 5$) for QC_1, QC_2, QC_3, and QC_4, respectively.

Table 1
Validation results of EtG in hair.

(a) Response function [7–333 pg/mg hair] ($k = 6$, $n = 2$, $p = 3$)			
	Day 1	Day 2	Day 3
Slope	0.0480	0.0489	0.0445
Intercept	0.0051	0.0092	0.0049
r^2	0.9964	0.9915	0.9966
(b) Linearity ($k = 4$, $n = 4$, $p = 3$) and LLOQ (pg/mg hair)			
Range: [8.4–259.4 pg/mg hair]			
Slope			1.0141
Intercept			−0.0454
r^2			0.9999
LLOQ			8.4
(c) Trueness ($k = 4$, $n = 4$, $p = 3$) (relative bias %)			
Levels (pg/mg hair)			
8.4			−4.6
40.8			11.2
130.4			3.9
259.4			6.5
(d) Precision ($k = 4$, $n = 4$, $p = 3$) (RSD%)			
Levels (pg/mg hair)	Repeatability		Intermediate precision
8.4	7.0		13.2
40.8	3.5		6.6
130.4	1.6		1.8
259.4	1.8		2.0

k represent the number of concentration levels, n represent the number of repetitions by levels, and p represent the number of non-consecutive days.

3.5. Trueness

The trueness, also called bias, expresses the closeness between the experimental mean value and the accepted reference value [21]. It indicates systematic errors and is expressed as percent deviation from the accepted reference value. The true value of each QC level was established using the mean of the results obtained by a cross-validation including the 7 external laboratories (see Section 3.4). As indicated in Table 1(c), trueness was found lower than the acceptance criteria (within ± 15% of the accepted reference value and within ± 20% at LLOQ), and was therefore acceptable for the EtG determination.

3.6. Precision: repeatability and intermediate precision

The precision is expressed from multiple analyses of the same sample at different days. It is expressed by the relative standard deviation (RSD%). The precision was evaluated by calculating repeatability (intra-day precision) and intermediate precision (inter-day precision) at each QC concentration level [22]. The pre-

Table 2
EtG concentrations (pg/mg) in QC_1, QC_2, QC_3, and QC_4 obtained by a quality control with external laboratories and resulting lower and upper confidence limits of the mean calculated with a risk of 5%.

Quality controls	Average (pg/mg hair)	Lower limit	Upper limit
QC 1	259.4	208.5	310.3
QC 2	130.4	109.1	151.7
QC 3	40.8	34.1	47.5
QC 4	8.4	7.7	9.1

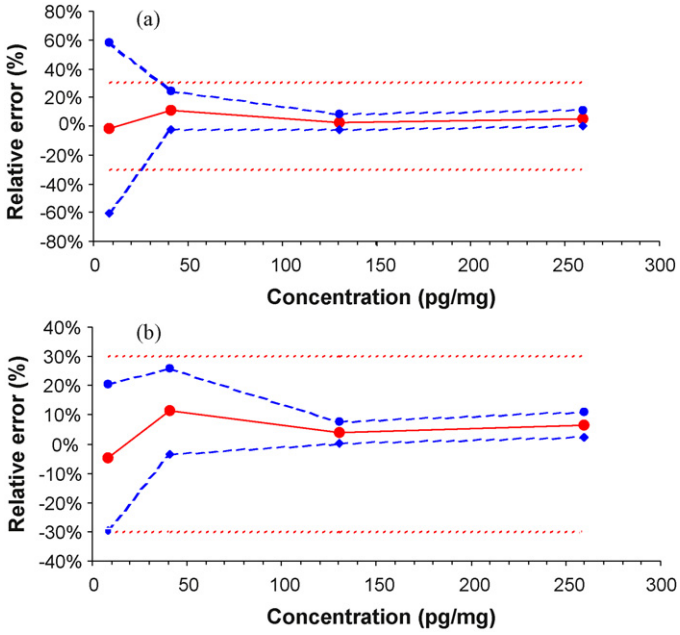

Fig. 3. EtG accuracy profile using a simple linear regression model (a) and a weighted ($1/x^2$) linear regression (b) within a range of 8.4–259.4 pg/mg hair. The continuous line represents the trueness (bias), the dashed lines are the lower and the upper accuracy limits in relative values. The acceptance limits have been set at ±30%.

cision indicates random errors. A statistical treatment based on ANOVA was applied to the obtained results with the measurement day as varying factor. The repeatability variance was estimated by measuring intra-days variance (S_r^2) and the intermediate precision variance was determined by adding intra- and inter-days variances (S_{IP}^2) [17,21]. As shown in Table 1d, the RSD values for repeatability and intermediate precision ranged between 1.6 and 13.2%.

3.7. Accuracy and lower limit of quantification

The accuracy represents the total error defined by the sum of systematic error (trueness) and random error (precision) [23]. The accuracy profile of EtG was established according to the proposals of the SFSTP for the harmonization of analytical method validation [24] and is illustrated in Fig. 3. The diagram demonstrates the capability of the analytical method to provide an analytical result considering systematic and random errors with a risk $\alpha = 5\%$ at each concentration level [17]. As a result, the calculated single measurement uncertainty was 25.1, 14.7, 3.8 and 4.3% for QC_4, QC_3, QC_2, and QC_1, respectivly. The mean bias (%) confidence interval limits for the QC levels were within the ±30% acceptability limits usually admitted by forensic Swiss laboratories [25]. Consequently, the lower limit of quantification (LLOQ) was set to 8.4 pg/mg hair. The method is considered accurate within the concentration range from 8.4 to 259.4 pg/mg.

3.8. Limit of detection

The LOD was evaluated from the signal to noise ratio S/N > 3. The noise was estimated measuring different hair samples of teetotalers without any EtG response. Using 30 mg hair, the LOD was estimated at 3 pg/mg.

3.9. Solid-phase extraction recovery

The SPE recovery was determined in triplicate at 3 concentration levels: 13 (low), 133 (medium) and 233 pg/mg hair (high). For each concentration, 3 blank hair samples were spiked with appropriate amounts of EtG and extracted as described in Section 2.7. Three other blank hair samples spiked with EtG after the elution stage were used as control. Recovery was calculated from the peak area ratios (in percent) between extraction samples and control samples at each EtG concentration. The used Oasis MAX (3 ml, 60 mg) cartridges combined anion exchange and reverse phase interactions as a result of a retention on a HLB (divinylbenzene-co-N-vinylpyrrolidone) copolymer modified by introducing a quaternary amine (aminopropyl). Because of its acidic properties, EtG should mainly be retained by ionic interactions. That allows a washing step with methanol removing interferences compounds. Methanolic elution of EtG in acidic conditions (2% HCOOH) enables fast evaporation of the solvent in comparison to published methods where aqueous solutions have been evaporated [6,8,10]. SPE recoveries for EtG spiked in hair at 3 concentration levels were between 39 and 69% (see Table 3).

Table 3
SPE recoveries (%) for EtG on Oasis MAX determined at 3 concentration levels (13, 133 and 233 pg/mg hair).

EtG concentration (pg/mg hair)	Recovery (%) ($n = 3$)
13 pg/mg	49.4
	66.6
	41.6
133 pg/mg	38.6
	69.0
	46.5
233 pg/mg	66.0
	56.9
	50.7

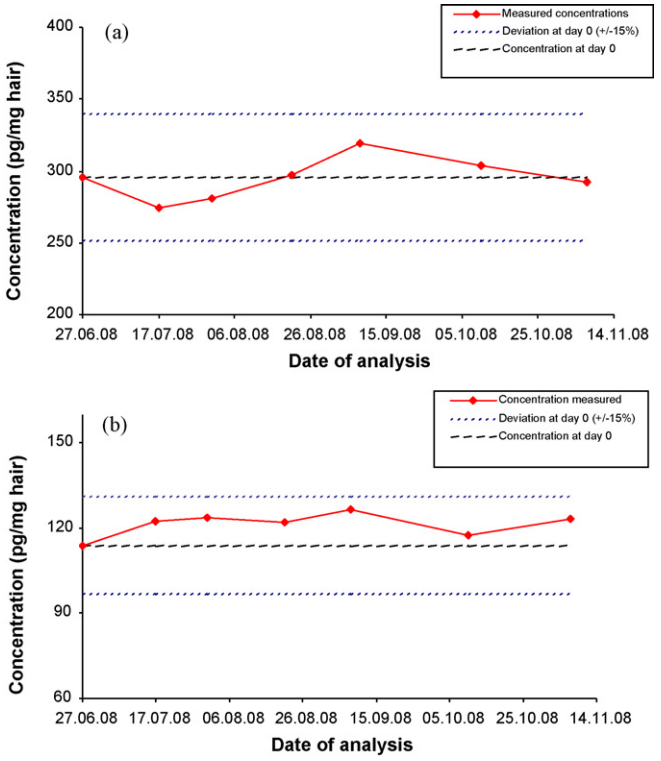

Fig. 4. EtG stability in hair from 2 autopsy cases over a period of 4 months (a and b). Hair powder was kept at room temperature in the dark.

Table 4
Quantification of EtG in hair (last 3–4 months) of seven autopsy cases with an alcoholism history (anamnesis) and/or pathologies caused by alcohol and revealed by macroscopic and microscopic autopsy findings.

Case number	Sex/age	Anamnesis	Cause of death	Autopsy finding	EtG in hair (pg/mg)[a]
A 01	M/39	Chronic alcoholic	Pulmonary embolism	Steatosis	80
A 02	F/49		Drowning	Steatosis/cirrhosis	679
A 03	M/55		Aortic anevrysm	Steatosis	273
A 04	F/69	Alcoholic/acute ethanol intoxication	Cervical trauma	Steatosis	712
A 05	F/46	Chronic alcoholic	Acute intoxication-trimipramine		61
A 06	F/67	Chronic alcoholic	Sudden death	Jaundice/ascites/cirrhosis	819
A 07	M/48	Chronic alcoholic	Digestive haemorrhage	Jaundice/ascites/cirrhosis	538

[a] Investigated hair length 0–3 cm. Smaller amount of hair sample were analyzed when EtG concentrations were above the upper limit of quantification (ULOQ).

3.10. Assessment of stability

3.10.1. Processed sample stability

To assess EtG stability in processed samples over short and long periods of time (intra-run stability and inter-run stability), we used derivatized extracts obtained from spiked blank hair samples of low (17 pg/mg hair) and high (167 pg/mg hair) concentrations. For each concentration, triplicates were stored at −20 °C in a refrigerator and at room temperature on the autosampler and analyzed after 0, 1, 4 and 8 days. The stability of EtG derivatives was assessed by comparing the measured concentrations at 1, 4 and 8 days with the comparison samples determined on day 0 [26]. The stability was deemed acceptable if the ratio (in percent) between the mean value combined low and high concentrations and the mean value of the comparison samples were within ±20%. As a result, EtG was stable (4–7% decrease) in hexane for 8 days indicating that the extracts can be processed within this period of time when samples were stored at −20 °C. In contrast, EtG was only stable for day 1 (13% decrease) at room temperature, and decreasing by 27%, and 31% at 4 and 8 days, respectively. These results indicate that EtG is unstable after the day 1 in derivatized extracts maintained at room temperature.

3.10.2. EtG stability in hair

To evaluate the EtG stability in hair, powdered hair samples from 2 autopsy cases with a known history of alcoholism were stored in the dark at room temperature and analyzed over 4 months. The stability was considered acceptable if the back calculated result did not deviate more than 15% from day 0. Stability study results are presented in Fig. 4. EtG was stable in the 2 samples suggesting that EtG is stable in hair matrix during several months when stored in the dark at room temperature.

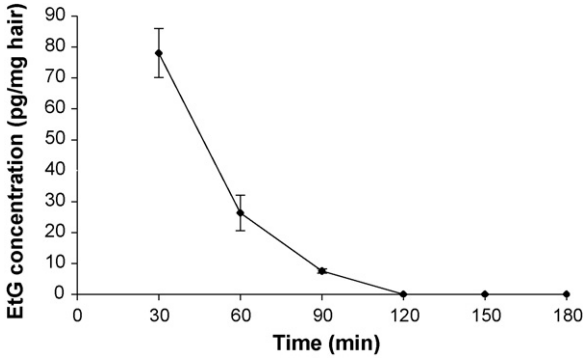

Fig. 5. Kinetic of aqueous ultrasonic (U.S.) extraction of EtG determined by the measurement of the residual EtG concentration (pg/mg) in real hair samples vs. the U.S. extraction time (min). Error marks indicate the standard deviation (SD) for 3 measurements from the mean value.

3.11. Kinetic of EtG extraction from hair

The kinetic of the EtG extraction was determined by analyzing 30 mg hair powder in triplicate from one autopsy case with a known history of alcoholism. At $t = 0$ min, 1 ml H_2O was added to 30 mg hair powder and the sample was then sonicated. At regular time intervals (every 30 min from $t = 30$ to 180 min), the supernatant was transferred to another tube and spiked with 20 ng EtG-d_5 for the EtG quantification. The procedure was repeated 5 times. The results indicate that a 2-h period was sufficient to extract the totality of EtG contained in the hair matrix as demonstrated in Fig. 5.

3.12. Analyses of real cases

The validated GC–NCI-MS/MS was applied to real cases. Table 4 shows the results obtained for 7 hair samples taken from autopsy cases performed at the University Center of Legal Medicine in Lausanne. EtG in hair was detected in all autopsy cases with concentrations ranged from 60 to 820 pg/mg.

4. Conclusion

In this study, a selective and sensitive identification and quantification method for EtG in hair was developed and validated using GC–MS/MS in NCI mode. The method was validated according to the guidelines of the French Society of Pharmaceutical Sciences and Techniques (SFSTP). In order to avoid a bias related to the EtG incorporation, the validation was performed using QC samples prepared with real hair samples from 2 subjects with a known history of alcoholism. EtG concentrations for each QC level were cross-checked by external laboratories. The method has proved to be accurate and reliable within an EtG concentration range from 8.4 to 259.4 pg/mg hair. This range includes the proposed cutoff values for the detection of heavy alcohol consumption [27–29]. The applicability of this method has been evaluated on several real cases with a known history heavy alcohol consumers. In these cases, the EtG concentrations ranged from 60 to 820 pg/mg hair.

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